PREFACE

The Institute of Medicine estimates that more than 4 million poisonings occur annually in the United States (Institute of Medicine 2004). In 2001, 30,800 deaths placed poisoning as the second leading cause of injury-related death behind automobile accidents (42,433 deaths) (Institute of Medicine 2004). In order to ensure that all potentially hazardous substances have proper warning labels, regulatory agencies require determination of acute toxicity hazard potential of substances and products. This determination for oral acute toxicity hazard is currently made using a test that requires laboratory rats. Historically, lethality estimated by the LD_{50} (i.e., the dose of a test substance that produces death in 50% of the animals tested) has been a primary toxicological endpoint in acute toxicity tests.

The conventional LD_{50} acute oral toxicity *in vivo* test method has been modified in various ways to reduce and refine[1] animal use in toxicity testing (OECD 2001a, c, d; EPA 2002a). Most recently, the LD_{50} was replaced, for hazard classification testing purposes, with the UDP, based on an Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) technical evaluation and formal ICCVAM recommendations (ICCVAM 2000, 2001c). This method now reduces animal use by over 70% compared to the previous method.

In 1999, at the request of the U.S. Environmental Protection Agency (EPA) Office of Pesticides, Prevention, and Toxic Substances, ICCVAM reviewed the validation status of *in vitro* methods for estimating acute oral toxicity. This request was based on studies published in recent years that showed a correlation between *in vitro* and *in vivo* acute toxicity. *In vitro* cytotoxicity methods have been evaluated as another means to reduce and refine the use of animals and these methods may be helpful in predicting *in vivo* acute toxicity. Since moving the starting dose closer to the LD_{50} reduces the number of animals necessary for the acute

[1] A reduction alternative is a new or modified test method that reduces the number of animals required. A refinement alternative is a new or modified test method that refines procedures to lessen or eliminate pain or distress in animals or enhances animal well-being (ICCVAM 2003).

oral systemic toxicity test, the use of *in vitro* cytotoxicity assays to predict a starting dose close to the LD_{50} may reduce animal use.

In October of 2000, the International Workshop on *In Vitro* Methods for Assessing Acute Systemic Toxicity sponsored by the National Toxicology Program (NTP), the National Institute of Environmental Health Sciences (NIEHS) and the EPA was convened in Arlington, VA. The Organizing Committee invited 33 expert scientists from academia, industry, and government agencies to participate in the Workshop. Invited scientific experts and ICCVAM agency scientists were assigned to one of four Breakout Groups and prepared recommendations on the following:

- *In Vitro* Screening Methods for Assessing Acute Toxicity
- *In Vitro* Methods for Toxicokinetic Determinations
- *In Vitro* Methods for Predicting Organ Specific Toxicity
- Chemical Data Sets for Validation of *In Vitro* Acute Toxicity Test Methods

Workshop participants concluded that none of the proposed *in vitro* methods had been formally evaluated for reliability and relevance, and that their usefulness and limitations for generating information to meet regulatory requirements for acute toxicity testing had not been adequately assessed. However, an *in vitro* approach proposed by the German Center for Documentation and Evaluation of Alternative Methods to Animal Experiments (ZEBET) was recommended for rapid adoption so that data could be generated to establish its usefulness with a large number of chemicals (ICCVAM 2001a). In addition, a separate *Guidance Document on Using In Vitro Data to Estimate In Vivo Starting Doses for Acute Toxicity* (ICCVAM 2001b) was prepared to provide sample cytotoxicity protocols and instructions for using *in vitro* data to predict starting doses for acute *in vivo* systemic toxicity tests.

ICCVAM, which is charged with coordinating the technical evaluations of new, revised, and alternative test methods with regulatory applicability (ICCVAM Authorization Act of 2000, Public Law 106-545; available: http://iccvam.niehs.nih.gov/about/PL106545.pdf), agreed that *in vitro* basal cytotoxicity test methods should have a high priority for evaluation. The

National Toxicology Program (NTP) Center for the Evaluation of Alternative Toxicological Methods (NICEATM) collaborated with the European Center for the Validation of Alternative Methods (ECVAM), a component of the European Commission's Joint Research Centre, to further characterize the usefulness of *in vitro* cytotoxicity assays as predictors of starting doses for acute oral lethality assays. NICEATM and ECVAM designed a multi-laboratory validation study to evaluate the performance of two standardized *in vitro* basal cytotoxicity test methods using 72 reference substances with the ZEBET approach of using the Registry of Cytotoxicity (RC) regression model. Based on the procedures described in the *Guidance Document* (ICCVAM 2001b), the validation study used two mammalian cell types (i.e., BALB/c 3T3 mouse fibroblasts [3T3] and a primary normal human epidermal keratinocytes [NHK]) for *in vitro* basal cytotoxicity test methods with a neutral red uptake (NRU) cell viability endpoint to predict starting for acute oral systemic toxicity test methods. The inclusion of human cells in the validation study also implements another workshop recommendation, that of evaluating whether cytotoxicity in human or rodent cells can be used to predict human acute toxicity.

The objectives identified for the validation study were to:

- further standardize and optimize two *in vitro* NRU cytotoxicity protocols using 3T3 cells or NHK cells in order to maximize intra- and inter-laboratory reproducibility
- refine the prediction model drawn from the ZEBET approach
- assess the accuracy of the two standardized *in vitro* basal cytotoxicity test methods for estimating rodent oral LD_{50} values across the five Globally Harmonized System of Classification and Labelling of Chemicals (GHS; UN 2005) categories of acute oral toxicity as well as unclassified toxicities and estimating human lethal serum concentrations
- estimate the reduction and refinement in animal use achievable from using *in vitro* basal cytotoxicity assays as one of the factors of the weight-of-evidence to identify starting doses for specific *in vivo* acute toxicity tests

- generate high quality *in vivo* lethality and *in vitro* cytotoxicity databases that can be used to support the investigation of other *in vitro* test methods necessary to improve the prediction of acute systemic toxicity

Scientists assembled for the ICCVAM-sponsored scientific peer review panel meeting ("Panel") on May 23, 2006 will independently assess the usefulness and limitations of the *in vitro* basal cytotoxicity test methods to predict starting doses for acute oral systemic toxicity test methods. The Background Review Document (BRD) on the two *in vitro* NRU test methods prepared by NICEATM and provided to the peer review panel and the public contains:

1. comprehensive summaries of the data generated in the validation study
2. an analysis of the accuracy and reliability of the test method protocols
3. related information characterizing the potential animal savings produced by using the *in vitro* basal cytotoxicity test methods as adjuncts to specific acute systemic toxicity test methods

The Panel will also evaluate draft test method performance standards, protocols, and draft ICCVAM recommendations. The public is invited to provide comments on the BRD and other documents and to attend the Panel meeting. Prior to this meeting, any public comments provided about the documents will be provided to the Panel for their consideration. The BRD can be obtained from the ICCVAM/NICEATM Web site (http://iccvam.niehs.nih.gov) or by contacting NICEATM.

Following the conclusion of the Panel meeting, the ICCVAM and its Acute Toxicity Working Group (ATWG) will consider the Panel report, the performance standards for the use of *in vitro* basal cytotoxicity test methods to predict starting doses for acute systemic toxicity test methods, and any public comments in preparing its final test method recommendations for these *in vitro* basal cytotoxicity test methods. These recommendations will be made available to the public and provided to the U.S. Federal agencies for consideration, in accordance with the ICCVAM Authorization Act of 2000 (Public Law 106-545).

On behalf of the ICCVAM, we gratefully acknowledge the many contributions of all who participated in the *in vitro* cytotoxicity validation study and those who assisted in the preparation of the documents evaluated at the peer review meeting. We extend a special thanks to the participating laboratory Study Directors and scientists who worked diligently to provided critical data and information. We also thank the ECVAM scientists who participated in the management of the validation study and who provided valuable information, comments, and opinions throughout the study. The efforts of the ATWG members were instrumental in assuring a complete and informative BRD. The efforts of the NICEATM staff in coordinating the validation study, providing timely distribution of information, and preparing the various documents are acknowledged and appreciated. We especially acknowledge Dr. Judy Strickland and Mr. Michael Paris for their coordination of the validation study and preparation of the BRD and other documents.

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